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Structures/textures of living/fossil microbialites and their implications in biogenicity, An astrobiological point of view

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ABSTRACT

Atacama's microbialites, able to live in such extreme environment, are possible candidates as models for searching life in other planets or moons. At present, little is known about their microstructure and composition. This study analyzes, mainly, the terrestrial microstructures in a dimensional field longer than 0.1 mm, through an original approach using photographs and macro pictures, appropriately magnified and consequently little defocused, in comparison with analogous images shot by NASA Rovers on Martian outcroppings. A method able to permit comparison of structures and textures of terrestrial microbialites to the microscopic photo images (MI) shot by the cameras mounted on the NASA rovers that since more ten years are present on the Red Planet (Opportunity, Spirit, Curiosity). The study highlights occurrence of widespread structures like microspherules (or clots), often organized into some higher order settings, such as donuts, polyspherules, filaments and, above all, intertwined filaments of microspherules, all showing features of an imperfect geometrical repetitiveness. The structural analysis has been connected with a textural study by a multifractal analysis, that is able to distinguish terrestrial biogenic stromatolites from abiogenic pseudo-stromatolites, and giving us a tool that might be applied for astrobiological purposes.

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KEYWORDS

Atacama;
Microbialites;
Mars;
Microstructural parallels;
Multifractal analysis;
Extreme environment.

INTRODUCTION

Life as we know is related to the chemistry of

carbon, the presence of liquid water and a source of energy, chemical or solar.^[1] The origin, evolution and distribution of life in the Universe are the ob-

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ject of astrobiology, a young discipline originally also known as exobiology, a term coined by NASA at the end of the 50's, with the objective of finding extraterrestrial life, notably on Mars^[2].

In recent years, astrobiologists, in an attempt to understand the origin and the evolution of life through the study of microbial communities in extreme environments on Earth, found that the extent to which terrestrial life can exist is much more extensive and the diversity of microbial life on Earth is far higher than scientists have believed so far. The discovery of extreme environments and organisms, able to colonize these, extends our perception of the tenacity of life and of the physical and chemical limits that it can support, making more convincing the search for life beyond Earth.

The fact that life forms have been discovered in areas considered inhospitable underlies the use of root "extreme" (from Latin *extremus*: "outermost, farthest"). The existence of extremophiles on Earth is a prerequisite for the scientific research of life forms on other planets, such as Mars or on the moons of Jupiter and Saturn, but also on planetary bodies around other stars.

All hyperthermophiles are *Archaea* or *Eubacteria* but Eukaryotes are common among psychrophiles, acidophiles, alcalophiles, piezophiles, xerophiles and halophiles, that respectively thrive at low temperatures, low pH, high pH and under the extremes of pressure, drought and salinity (for an overview see: <http://www.astrobiology.com/extreme.html>)^[3].

The persistence of life forms in extreme terrestrial environments, such as terrestrial analogues of Mars (the Dry Valleys of Antarctica and the hyper-arid core of the Atacama Desert) provides a prerequisite for the scientific search for life beyond Earth. At the same time the exposure of terrestrial extremophile organisms in Low-Earth Orbit (LEO) space platforms, provided for example by the European Space Agency (BIOPAN and EXPOSE), contribute to our understanding of the tenacity of life as we know it^[4].

Among extreme-tolerant organisms, the so called anhydrobiotes have evolved features to cope with desiccation^[5]. They show a high survival rate to such a stress, colonizing extreme environments charac-

terized by prolonged periods of drought and revive upon rehydration by preventing desiccation-induced damage and/or by repairing it after rewetting^[6]. In extremely hot and cold deserts, where life is placed at the limit due to low humidity and temperature values, the presence of cyanobacteria have been frequently reported^[7,8]. Unicellular cyanobacteria of the genus *Chroococcidiopsis* have been recorded in hot and cold deserts worldwide where, depending on geological conditions, they colonize either microscopic fissures (chasmoendoliths) or structural cavities (cryptoendoliths) of rocks or form biofilms at the stone-soil interface under pebbles of desert pavements (hypoliths)^[9,10]. Thanks to their ability to survive near the absolute physical limits for life processes, desert cyanobacteria are studied as part of astrobiological researches for searching life on Mars^[11].

We have investigated structures and textures of some terrestrial microbialites comparing their patterns with images, acquired by Opportunity Mars Rover, on the laminated sediment of Meridiani Planum. This morphological study is supplemented by a multifractal in order to obtain effective biosignatures for the search of life on Mars. Our analysis is supported, also, by the persistence of life forms in extreme terrestrial environments such as the Puna Andina high plain, a natural hot spot of microbial biodiversity which enable is to come back to the origin of life on Earth, but also to guess how could be life in other planets of our Solar system.

We present also images of microbialites ultra-structure, from living samples observed by Scanning Electron Microscope (Farias ME).

MATERIAL AND METHODS

Terrestrial and martian samples

The terrestrial samples analyzed in this study come from the Salar de Atacama depression (Figure 1) in northern Chile^[12]. In particular, the specimens, including ten macroshot from sampling at Llamara, eight from Socompa, seven from LaBrava and eight from Tebenquiche lagoons, were photographed and morphologically investigated, in particular, at the surface layers of the microbial mats. Each image, combined with a metric scale, has been scanned in a

net of rectangles of about 20 mm * 13 mm in dimensions. Then, every image has been transferred into a Microsoft Publish 2010 software and enlarged until seven times to study the existing textures in a dimensional field where are clearly evident microstructures of about 0.1-1 mm by length. Indeed, the enlargement vision has been pushed twice the previous frame, just below fluttering conditions. Finally, all the images have been analyzed in color and black and white shades to compare them to those of the Martian rover increasing, slightly, the contrast of about 10% - 20% for an optimal vision of the microstructures.

As regards Mars samplings, it has been undertaken a systematic analysis of black and white Microscopic Images (MI) obtained by Athena, a camera mounted on the NASA Mars Exploration Rover (MER) "Opportunity". The field of view of Athena is 1024*1024 pixels in size and its optics provide a square frame of 32 mm of-field sampling. at the working distance of about 63 mm from the front of the lens barrel to the object plane, consequently having a resolution of about 30 μ ^[13]. In these conditions, applying a low enlargement, of three times to the original MI images, microstructures arises if analyzed above 100 μ in diameter; these last corresponding at the lower shape limit, corresponding to about 9 Athena pixels. These images, characterized by constant dimensions, have been processed as the terrestrial ones (by Publish Software), enlarged by three-five times for sampling sub-frames and microstructural morphological comparison; and enlarged about three times the original images for multifractal analysis. Selected sub-frames were, slightly, increased in contrast of about 10% - 20%, to highlight micro-textures/structures to compare to living microbialites and fossil stromatolites.

Samples concerning stromatolites include 17 quoted images released by the WEB (one sample of which was a shot by Athena on Earth sampling, and two images of living colony of cianobacters) and 3 samples photographed at the Regional Museum of Natural Sciences, Turin. Previously, further photos of stromatolites from museum samples (University of Calabria, Italy) have been worked out with color images transformed in grey shades, to test image samplings at various conditions in order to standardize

the procedures.

Finally, fifteen images from samples of terrestrial abiogenic pseudostromatolites (speleothems: alabaster, amethyst geode, chalcedony, malachite, stalactite), synthetic pseudostromatolites, other granular sediments presenting microspherules (siltstone) were processed and analyzed using the same procedures as above.

Comparison was performed using adequate amplification on macro and normal photos in order to obtain the same dimensional range and similar resolution. In some cases, the use of macro photos allows us to analyze microstructures near 100 μ and smaller deepening same details, as for example, very thin and long filaments that may already represent microbial forms. On the contrary, above 100 μ in length, the use of macro photos in place of normal photos does not entail appreciable differences, showing similar textures at different scales. The employment of normal photos has let to compare MI images to those kept from a large record of museum stromatolites samples, also occurring on the web sites. Besides, the use of normal photos of living microbialites from Atacama allowed us to analyze structures/textures at the same scale and similar focusing of fossil stromatolites.

Fractal analysis

We have processed fifteen quoted images of fossil/living microbialites and ten images from samples of abiogenic pseudostromatolites (speleothems: alabaster, amethyst geode, chalcedony, malachite) and of synthetic pseudostromatolites. They have been acquired, magnified and worked, in order to obtain the same (\pm 10%) dimensional scale, resolution and acutance of the Athena's photos by the Martian Rovers. The contours presented in the images were automatically extracted and converted to single pixel outlines by a canny-edge filter (Digital Image Magnifier software by Strikos Nikolaos: <http://www.softoxi.com/digital-image-magnifier.html>). The obtained textures were then characterized by analyzing their geometrical complexity, entropy and algorithmic complexity.

To evaluate the geometrical complexity of the patterns, the local fractal dimension was measured using the box-counting algorithm. When our texture

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resulted multifractals, as identified by the two straight lines on the log-log plot the algorithm was applied for the two linear regions (200 - 10 pixels = 2 mm - 0.1 mm and 10 - 5 pixels = 0.1 mm - 0.05 mm) [14]. The existence of log-log straight lines ($p < 0.001$) justified the use of the fractal analysis, applied here as a tool to obtain the morphometric indexes. The method was validated by measuring computer-generated Euclidean and fractal shapes of known fractal dimensions.

To determine the algorithmic complexity ("randomness") of the patterns, relative Lempel-Ziv, L-Z, values were calculated according to the Kaspar and Schuster algorithm using the Chaos Data Analyzer version 2.1 software package (CDA; Pro, Academic Software Library, North Carolina State University, USA) over the vectorialized images [15]. Relative L-Z values is close to 0 for a deterministic equation, close to 1 for totally destructured random phenomena.

Tortuosity, or the fractal dimension of the minimum path, D_{min} , was computed for each cluster present in the image from the power law $I_c = r^{D_{min}}$, where D_{min} is the exponent that governs the dependence of the minimum path length between two points (I_c) on the Pythagorean distance r between them in a fractal random material. To obtain D_{min} , the maximum diameter and the half perimeter of the microstructures present in the textures were measured using an automated procedure (Image Pro Plus software, Media Cybernetics, USA). For each image 100-500 microstructures were measured. The slope of the log-log plot (maximum diameter vs. perimeter) represented D_{min} . The existence of a log-log straight line ($p < 0.001$) justified the use of the fractal analysis in order to obtain the morphometric index. The method was validated with the original one by Hermann and Stanley with a maximum shift of $\pm 3\%$ [16].

Statistical analysis

Mean intra- and inter-observer coefficients among the images varied $< 2.0\%$ and $< 3\%$, respectively.

Comparisons between groups were analyzed by Mann Whitney test; t-test was applied in order to verify the linearity of the log-log plots.

RESULTS

Microbial mats, microbialites and endoevaporites at Atacama. The study area.

The Salar de Atacama depression, in Puna Andina high plain, is a distinct geomorphologic structure in northern Chile and it is the oldest and the largest evaporitic basin in that country [12]. It is a tectonic structure (a Graben), forming a basin filled with Tertiary to Quaternary clastic and evaporitic sediments of continental origin. The Salar's hydrogeological setting is quite complex, receiving both surface and groundwater inputs predominantly from the east [17]. The Salar de Atacama comprises two main units: a core and a marginal zone. The core (1.100 km² of surface area and 900 m in thickness) consists of a porous halide (90%) impregnated with a sodium chloride brine rich in lithium (Li), potassium (K), magnesium (Mg) and boron (B) occupying the interstices of the halide. The marginal zone of the salt consists of thin saline sediments that are rich in sulfates, especially gypsum [17,18]. There are two types of brines in the Salar de Atacama: type Na-Ca-(Mg)-Cl ("calcium" brines) and other types of Na-(Mg)-SO₄-Cl ("sulfate" brines) [18]. Tebenquiche is located closer to the core and La Brava is in the marginal zone (Figure 1).

The main water input is through leaching and direct contributions of Tertiary and Quaternary volcanic material. In the lowest region of Atacama basin, groundwater surfaces form a series of lakes including: Laguna de Piedra, Laguna de Tebenquiche, Chaxas, Burro Muerto and La Brava [18]. The environmental conditions of these lakes are characterized by: (1) high solar radiation due to a lower barometric pressure at high altitude and consequently decreased absorption of solar radiation [19]; (2) extreme daily temperature fluctuations typical of desert environments; (3) net evaporation producing hypersaline water and (4) high arsenic concentrations in the water due to volcanic events [20]. All these conditions contribute to support an environment that selects for microbial extremophiles, interesting for the study of extraterrestrial parallels, as for Mars. In past years, microbial ecosystems, associated to mineral (MEAM) like microbialites, mats, biofilms and gypsum domes with endo-evaporitic communities

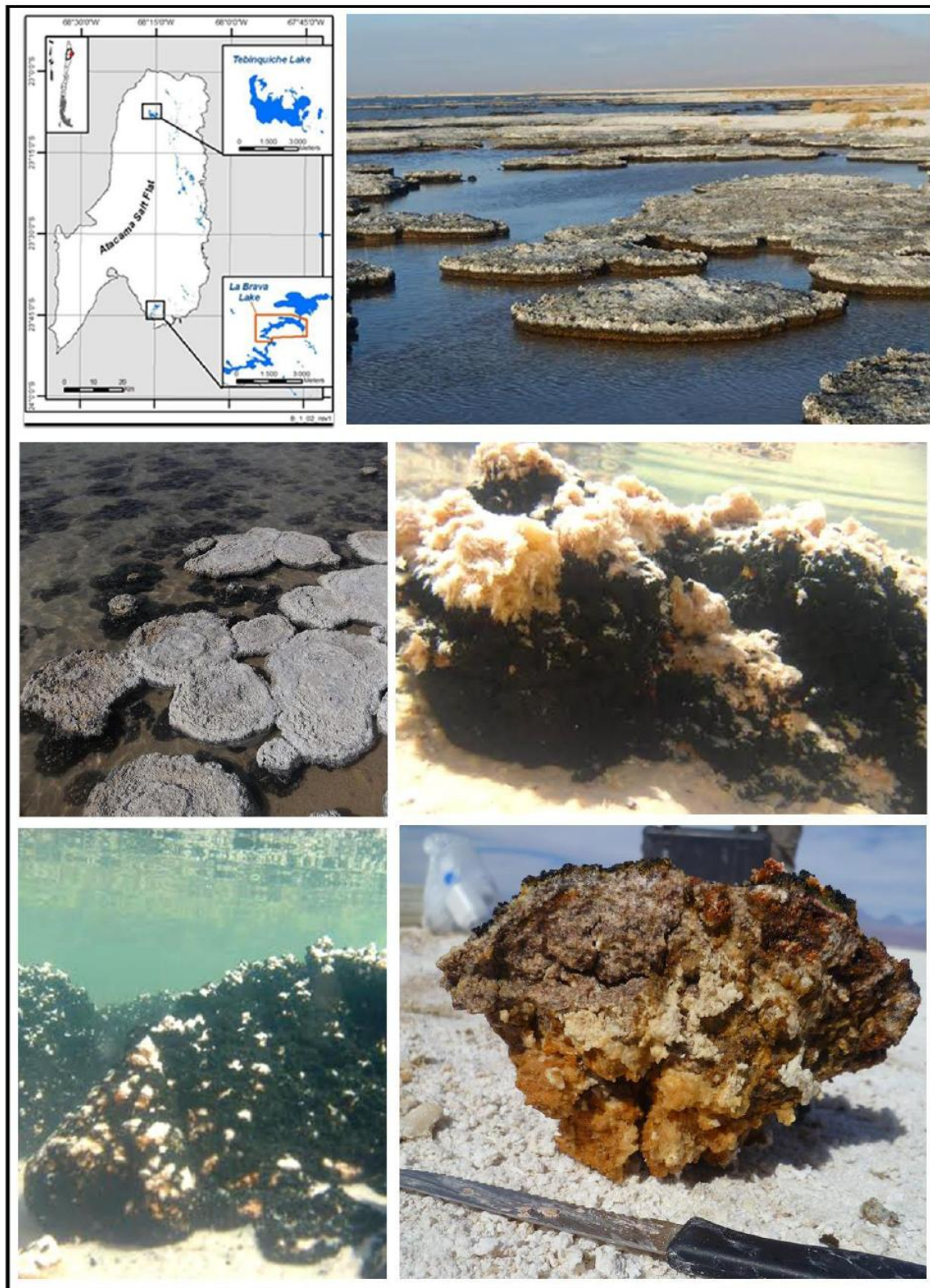


Figure 1 : Pictures show meso and macro structures occurring at salar de atacama and localization of la brava and tebenquiche lakes

have been reported in shores of high salinity lakes associated with evaporitic systems at Andean's High Plan^[21,22]. They were found associated with arago-

nite in stromatolites and oncolites at Socompa lagoon (3600 m asl) and at Negra lagoon (4600 m asl), respectively. They were, also, found in biofilms

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associated to Gaylussite at Diamante lagoon (4600 m asl), in halite-aragonite mats and aragonite-calcite microbialites at La Brava lagoon, in gypsum endo-evaporites and in aragonite-gypsum microbialites at Llamara, Laguna de Piedra, Tebenquiche and in microbial mats at Kiritimati Atoll in Central Pacific^[23-27].

Tebenquiche and La Brava lakes display a characteristic salinity gradient that results of ground and/or superficial water input and evaporation^[12]. Along these salinity gradients, different microbial ecosystems developed: organic-rich and non-lithifying microbial mats were found along the shoreline at low salinity (62 g/L) at Tebenquiche and, with increasing salinity (116 g/L), the amount of mineral precipitation and hence lithification increased as well. At La Brava the opposite situation was detected. Some mats were placed at the shore with higher salinity (119 g/L) whereas microbialites were found submerged in the lake with higher salinity (72 g/L) (e.g., from intermediate to hard mats)^[28]. The increasing amount of mineral incorporation in mats was confirmed by the increased mechanical resistance during deployment of microelectrodes. In the submerged zone at high salinity, extensive areas of hard domes thrive. The soft microbial mats have a vari-

ety of macroscopic morphologies including small domes with cerebral, snake and globular morphologies^[29-31]. In some areas, bulbous mats accumulate gas at the subsurface as highlighted in cross section. The submerged non-lithified mats have a typical pink appearance. However, when exposed to the air, a white evaporitic crust covers the surface. At the highest observed salinity (117 g/L), a hard domal structure forms. This area of maximum salinity and conductivity supports the formation of domal structures. In La Brava, the microbialites grow upward until they reach the water/air interface when they continue to spread laterally forming platforms. These microbialites display a typical sequence, proceeding from top to bottom, of white, green, purple and dark colored layers. XRD analyses revealed that Tebenquiche microbial mats comprised predominantly halite (42%) with minor contributions of calcite (22%), gypsum (22%) and aragonite (12%), while the domal evaporitic systems from Tebenquiche consisted entirely of gypsum^[27]. In La Brava, the mineralogical analyses of the microbial mats revealed halite (82%) as the major component and aragonite (13%) and calcite (7%) as the minor ones (Figure 2)^[28]. La Brava microbialites consisted exclusive of aragonite (CaCO₃). Microbial diver-

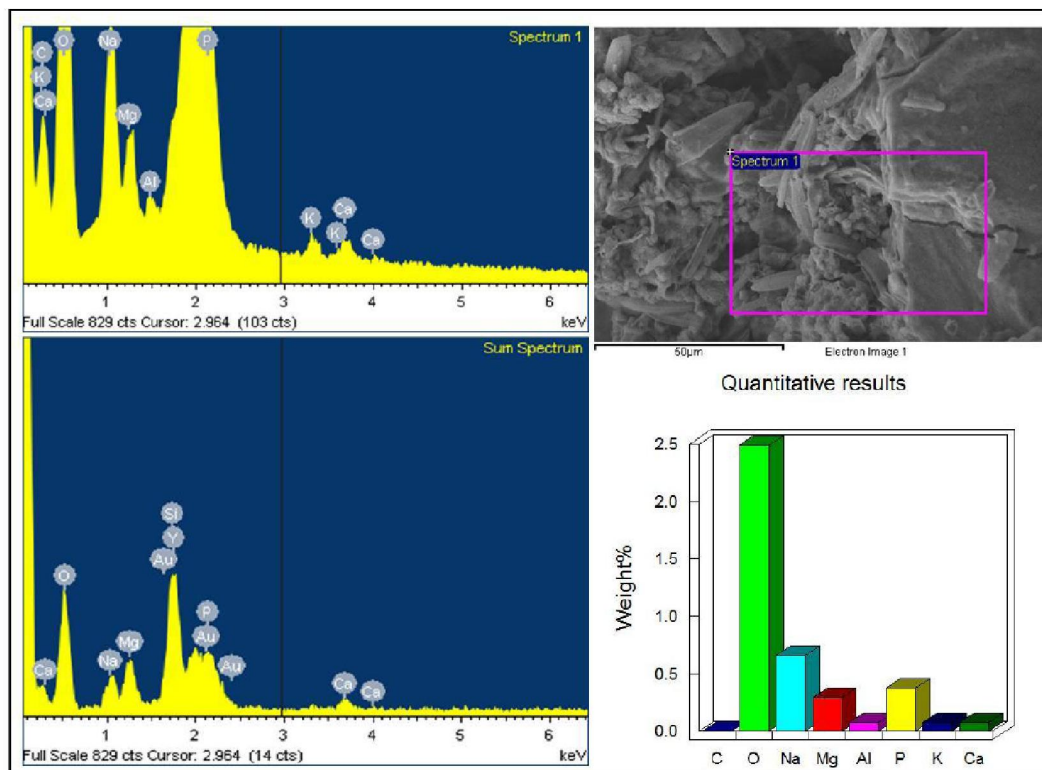


Figure 2 : Chemical composition of La Brava sample shown on figure 3

sity, described directly or indirectly by pigments analyses, V4 pirosequencing and Scanning Electron Images (SEM), demonstrated that Cyanobacteria are present in the top layers^[28]. As well, by SEM a widespread occurrence of spherulitic structures (clots) on could observe (Figs 3 and 4), made by a mix of organic (including diatom) and inorganic material, and set (or connected) side by side in elongate/crossing arrays, entrapped by Cyanobacteria filaments (Figure 3). The dimensions of more appreciable microspherules and their array in elongate bodies in

these SEM images are close to 0.1-0.3 mm.

More generally, microbialite are layered microbial communities made by spherulitic components that initiate in the photosynthetic dominated orange top layers and further grow in the green and purple layers below. Therefore, these spherulites are considered as the product of an extraordinary high photosynthetic effect simultaneous to a high inhibition by pristine exopolymers. Then, a successive heterotrophic bacterial activity leads to a condensation of the exopolymer framework and, finally, to

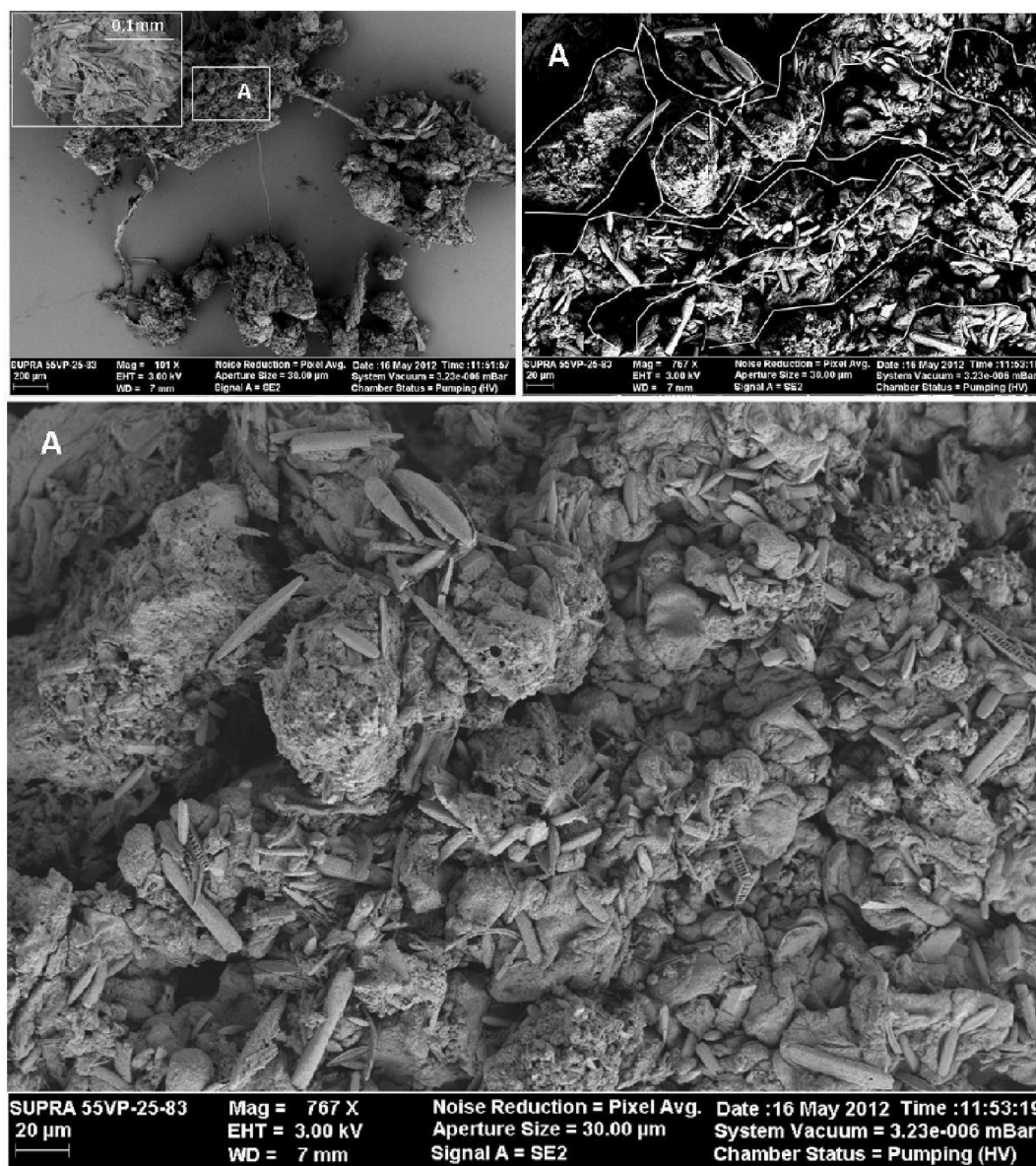


Figure 3 : Scanning Electron Images of a living microbialite at La Brava. Pictures highlighted occurrence of clots (subframe top on the left), set side by side along linear arrays (top on the right), here described as “polispherules filament structure”: a mix of filaments and microspherules of mineral origin and other living forms (diatoms are present) built by Cyanobacteria. Top on the right: structure like “filament of microspherules” are highlighted by a contrast increase, bottom, the original SEM image

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the formation of crevice-like zones partly degraded by exopolymers^[32]. Also in Kiritimati Atoll (Central Pacific) the microbial mat is associated with the formation of stratified microbialites composed by bacterial communities along a vertical stratification where the surface layers are dominated by aerobic microbes while the deeper ones harbor sulfate-reducing, purple sulfur and anaerobic bacteria^[27]. This microbial mat is embedded in Extracellular Polymeric Substances (EPS) secreted by the metabolism by a complex microbial community which shows a vertical distribution profile mainly driven by light and oxygen penetration. The stratification in layers, highlighted by a typical chromatic scale from orange, to green, to purple and /or grey colors^[32], displays a separation into three major mat zones proceeding from the surface to the bottom of the mat: the photic-oxic zone, the transition zone and the an-oxic zone, with specific bacterial communities^[33].

The studied samples of Atacama microbialites show surficial layers made by a tangle of translucent microspherules (Figures 4 and 5), having orange, yellow, brown, green, purple, grey, black, brown, and white colors.

Such microspherules, as they appear in the enlarged photo shot of fresh samples are generated by microbial activity and are rich of EPS. Occurrence of different pigmentation is a peculiarity not only of microbialite layers but also of microstructures, as are microspherules and their more complex arrays (Figure 5). During metabolism and as consequence of first diagenesis they will change composition, become enriched in biominerals and will joint in layers or composite masses, containing both organic and inorganic components as shown by the SEM images (Figure 3).

Structural features of microbialites and their implication for life searching.

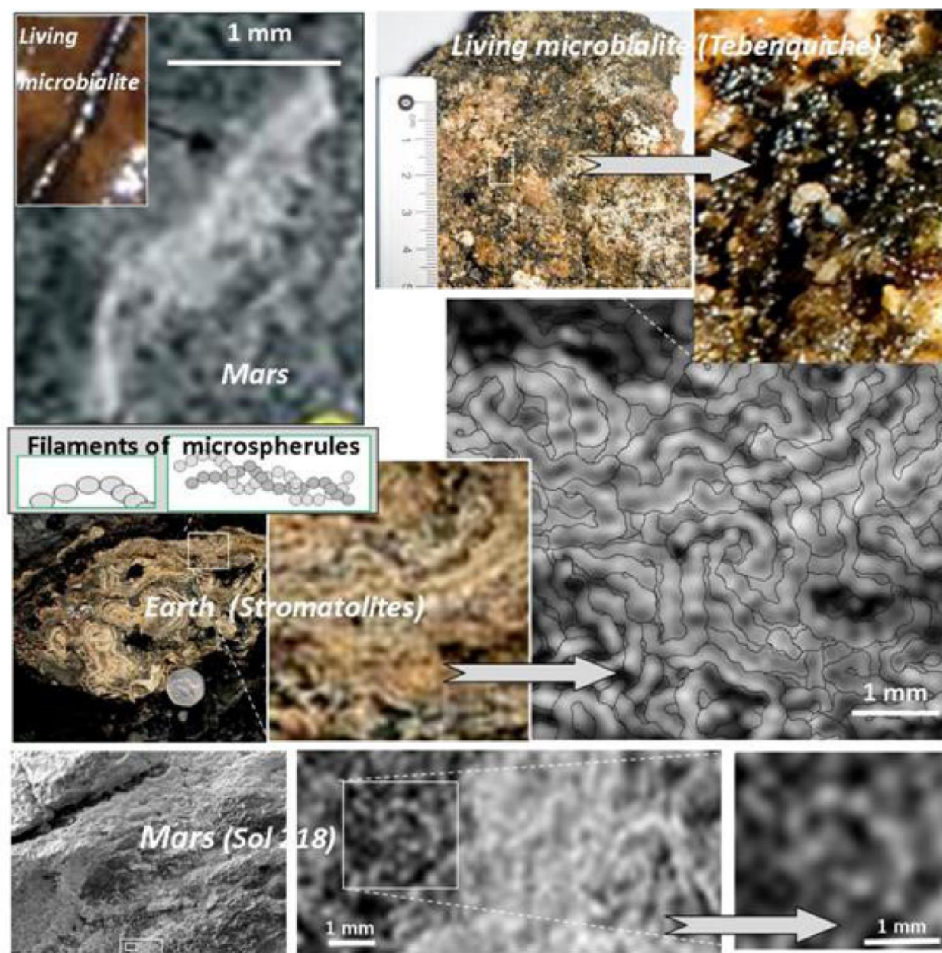


Figure 4 : Pictures show widespread occurrence in living microbialites, collected at la brava lagoon, made by a tangle of variously colored microspherules processed in order to obtain the same dimensional scale, resolution and acutance as the martian images obtained by the martian rovers (opportunity and spirit)

Microbialites, resulting from different pattern of bacterial activities, show peculiar and discriminant structures/textures at micro, meso and macro levels that could be used for their recognition^[34]. More in general, while skeletal components within a sediment are not significant, due to their external origin, fabric, as well known, is related to the sedimentary environment and it is very important for genesis understanding. By doing this way and considering the microscopic dimensions of microbes, the larger microstructures observed represent products of a well-known complex activity, made by the binding and

trapping activities of biomineralizing colonies, during deposition stage, metabolism and early diagenesis.

Our investigation shows that microstructures in microbialites consist of a tangle of microspheres having almost similar dimensions (0.1 - 0.3 mm) and various pigmentation. Such structures are well known in algal settings and fossil stromatolites, as peloid or ooids while their structural assembly into more complex arrays, set from 0.3 mm up to several millimeters, is little known^[35]. Figure 5 shows a set of sampling pictures of La Brava and Tebenquiche

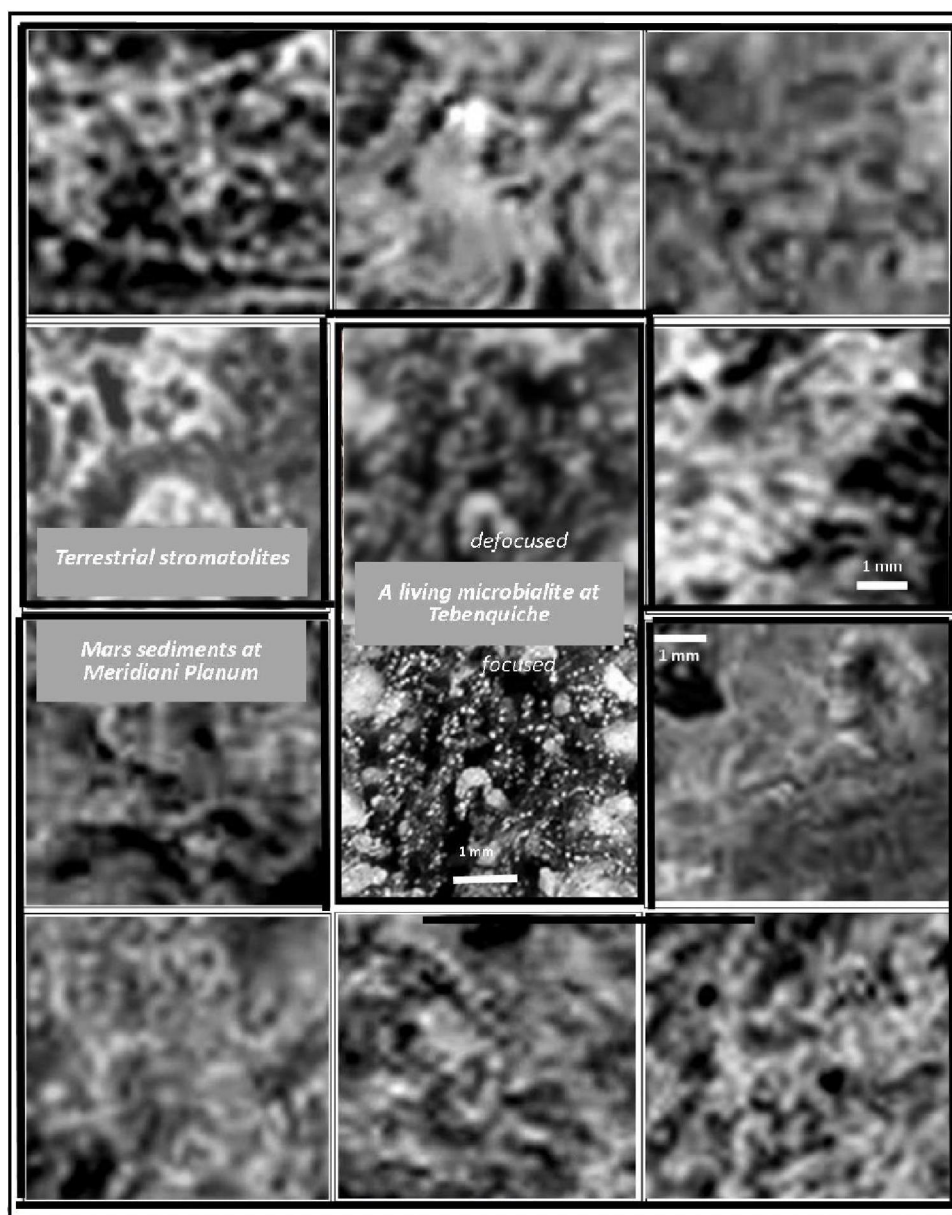


Figure 5 : Pictures show a set of typical textures, as are interwoven filaments of microspherules, polispherules and donuts, collected both in living microbialites from tebenquiche lagoon, as well in terrestrial stromatolites and Martian sediments. Images were processed in order to obtain the same dimensional scale, resolution and acutance as the Martian images obtained by the martian rovers (opportunity and spirit)

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microbialites, respectively related to normal and macro photos, where it is highlighted the most important/common microstructures, made by spherule arrays analyzed on the size ranging 0.05 - 2.0 mm, which are: donuts, polispherules, filaments of microspherules and interwoven filaments of microspherules. Similar structures were, also, found on stromatolites and on MI rover shots of Martians sediments at Meridiani Planum (Figures 5 and 6).

In particular, as regards the “donut” structure, observed in living microbialites, an item previously described as a basic pattern, forming on Mars lamina and the well-known “blueberries” (SB structure), it seems to be characterized by a central darker and sharper microsphere and -on living microbialites- by a shining halo containing other microspheres of similar size^[36]. As well, a rare similar structure on Mars shows a central larger spherule surrounded by

equidimensional and smaller ones.

As regards the “polispherule” structure, it is made by a polycentric settings of smaller spherules, having equal color and dimensions; somewhere their surface, reflecting internal setting, is lobate. Similar structures were supposed for Mars, in the “Blueberry Field” at Meridiani Planum. In particular, as regards the famous “blueberries”, it was pointed out; that these spherules could have two kind of structure: a) concentric, made by one or several encircling layers; b) polycentric, made by minor irregular microspherules, somewhere extruding and forming external polilobate shapes as bispherules, polispherules and/or flying saucers^[36,37].

As regards the filaments of microspherules, their occurrence is highlighted by their color and size uniformity, as shown in La Brava and Tebenquiche living microbialites.

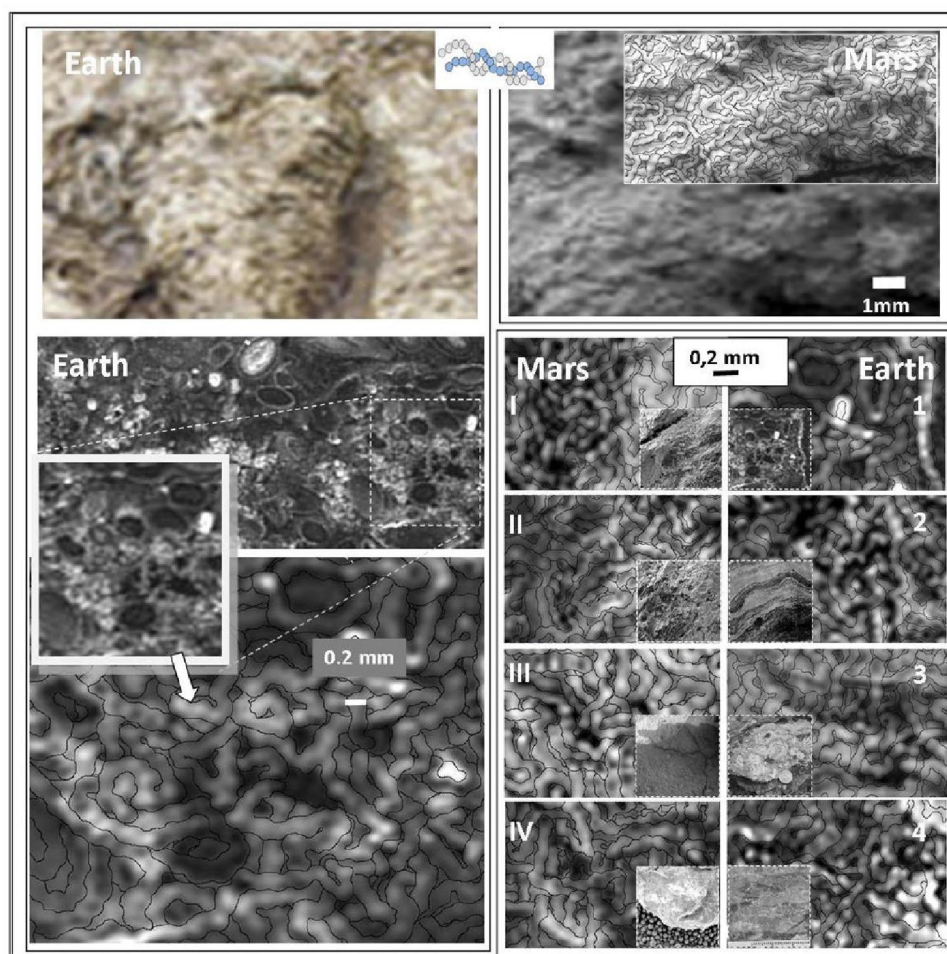


Figure 6 : Pictures showing similar textures of filaments of microspherules both for various stromatolite samplings, as well for Martian sediments (MI images of opportunity NASA rover). Images were processed in order to obtain the same dimensional scale, resolution and acutance as the martian images obtained by the martian rovers (opportunity and spirit). To see the “interwoven filaments of microspherules” at higher resolution and amplification see Figure 3

As regards the “interwoven filaments of microspherule”, such structures seem to be unknown in the literature, despite their presence could be observed both in living microbialites than in fossil stromatolites and in many enlarged pictures of Martian outcroppings (Figure 6).

In this dimensional system of microscopic structures, from 0.1 to same millimeters, made by few basic arrays essentially due to a spherical or linear aggregation, it can be seen local passages from ordered to chaotic systems and, more generally, from one structure to another. Polispherule somewhere

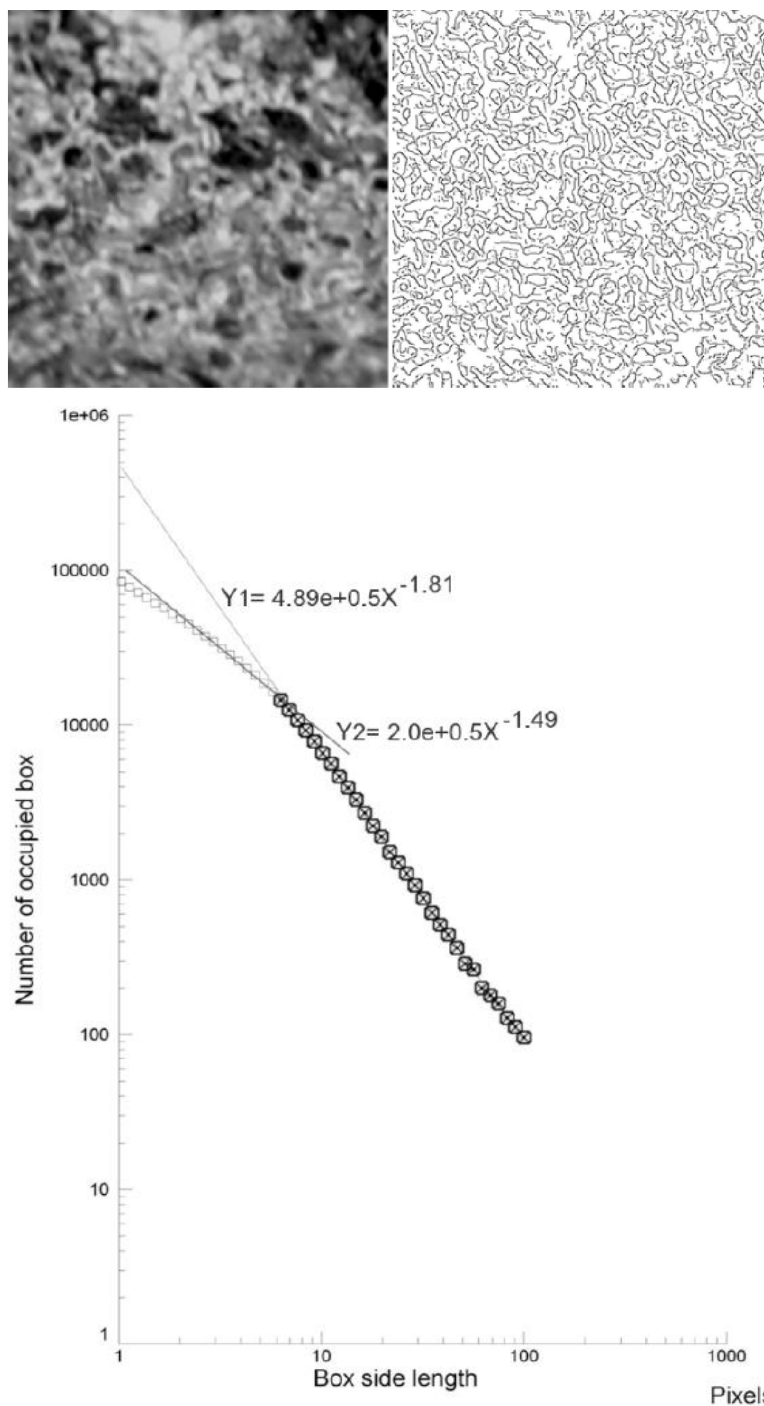


Figure 7 : Stromatolite, a multifractal texture. Biogenic stromatolite, a multifractal texture. The characteristic fabric made of microspherules and intertwined structures (top, left), its automatically extracted contours (top, right) and its log-log plots (bottom). Two lines are present in the log-log plot, performed in order to evaluate the geometric complexities of the sample (some hundreds of filaments/microspherules present in the microscopic field). The fractal dimensions, at high and low scales, are the slopes of the straight lines

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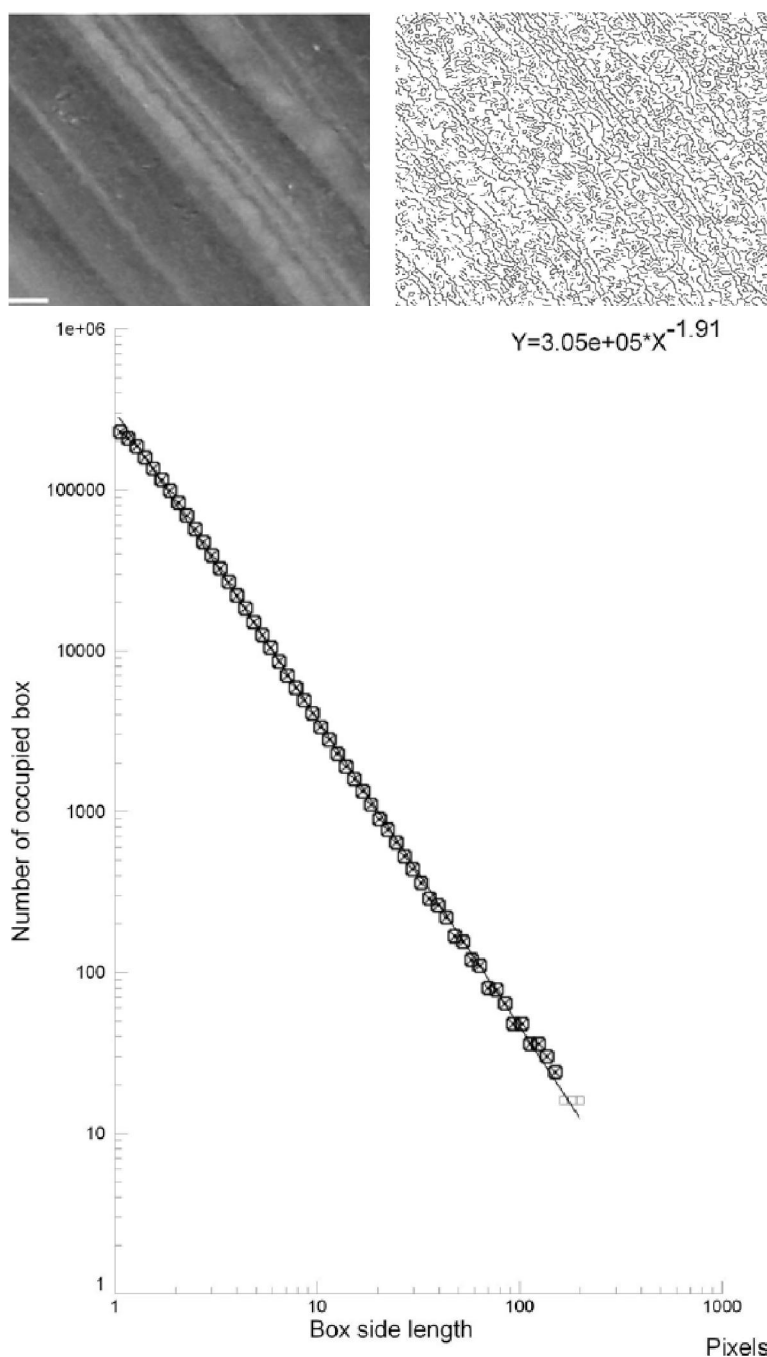


Figure 8 : Abiogenic pseudostromatolite (a stalactite). Stalactite (top, left), its automatically extracted contours (top, right) and its log-log plots (bottom). The log-log plots are fractals (one single straight line from 200 to 5 pixels, corresponding to 2 mm - 0.05 mm) but not multifractals like as the biogenic stromatolites (figure 7).

shows internal passages from a tangle of smaller chaotic microspheres (thrombolytic structure) to linear -set by set- arrays (laminated structure) or to intertwined filaments. In other cases, some donuts seem to become incubator of bigger polyspherules or forming -side by side- a lamina. In other conditions, microspherule filaments seem to modify their laminar assembly to give linear splitting or to become dendritic.

All these structures and aspects are very interesting and difficult to explain them as abiogenic artifacts. In fact, while the sediment structure follows universal and repetitive laws, depending upon a variety of physicochemical environments, the biological structure, within the same environment and at a microscopic scale, vary depending on the metabolic activity of living organisms^[38-43]. Then, it is important for searching life on Mars, not only to look for

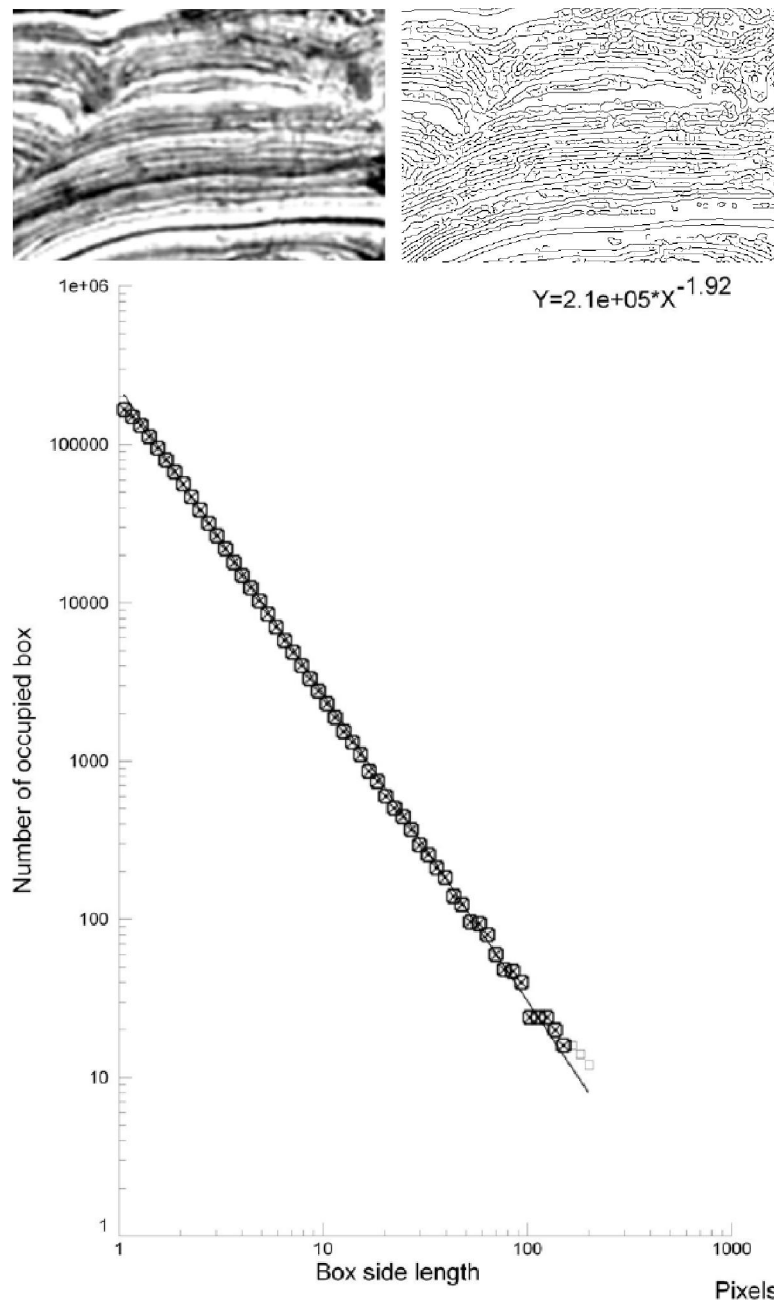


Figure 9 : Abiogenic (pseudo)stromatolite (a synthetic pseudostromatolite). Terrestrial synthetic (abiogenic) pseudostromatolite (top left), its automatically extracted contours (top right), its log-log plots (bottom). The log-log plots are fractals (one single straight line from 200 to 5 pixels, corresponding to 2 mm - 0.05 mm) but not multifractals like as the biogenic stromatolites (figure 7)

structural parallels with our ancestors, but also to search for the occurrence of possible discriminant biogenic factors (biomarkers) not explicable by chemical and physical rules^[44].

Stromatolites vs. pseudostromatolites, a fractal analysis.

Biogenic stromatolites present a multifractal structure (Figure 7) while abiogenic pseudostromatolites present a simple fractal structur,

natural or synthetic, as well granular sediments (Figures 8,9,10); on every tested length scale, the microstructure repeats itself perfectly, as indicated by the single linear log-log plot.

Biogenic stromatolites also present a lower geometrical complexity and entropy, and a lower randomness index (a more ordered texture) than those ones of abiogenic pseudostromatolites (<0.01, <0.001, TABLE 1).

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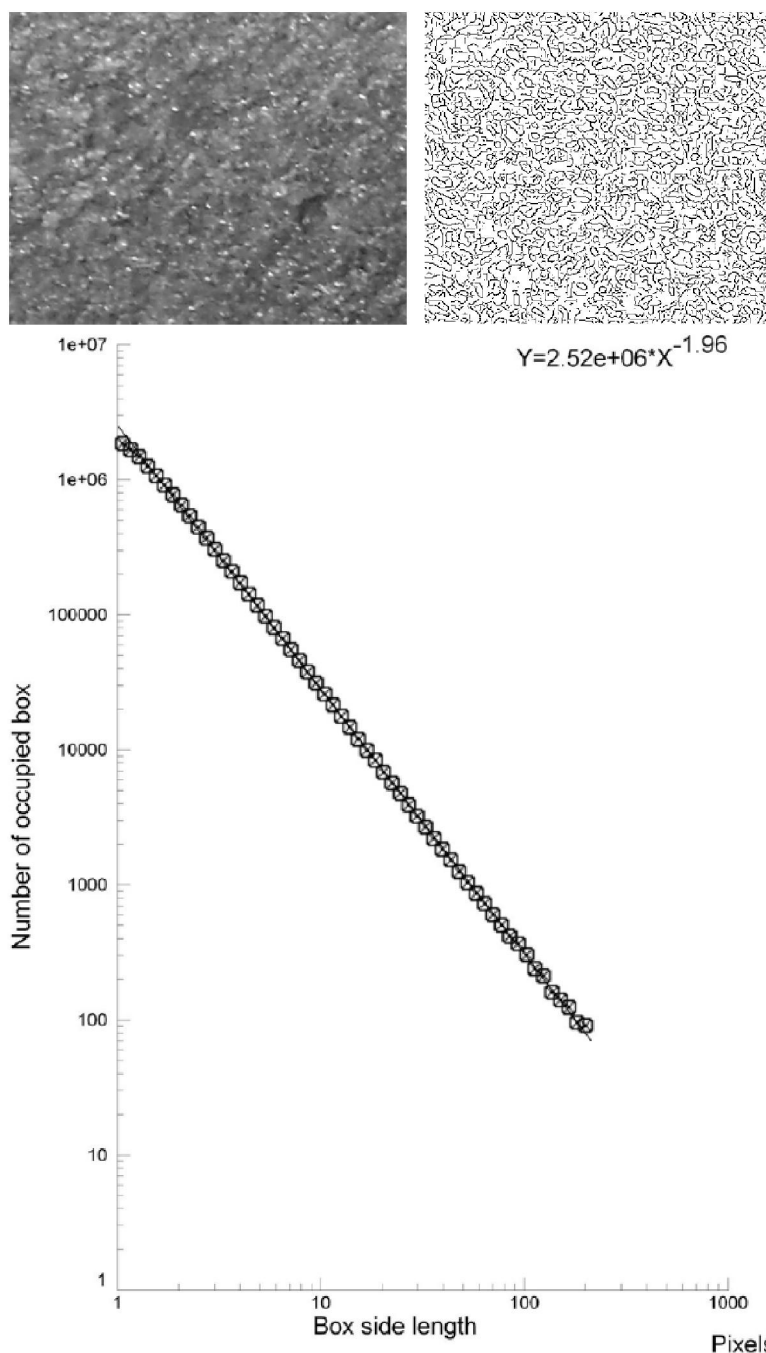


Figure 10 : Terrestrial granular microspherules-containing sediment (a siltstone). Terrestrial granular sediment showing microspherules (top, left), its automatically extracted contours (top right), its log-log plots (bottom). The characteristic fabric made of microspherules and intertwined structures present in biogenic stromatolites, is not present. The log-log plots are fractals (one single straight line from 200 to 5 pixels, corresponding to 2 mm - 0.05 mm) but not multifractals like as the biogenic stromatolites (Figure 7)

DISCUSSION AND CONCLUSIONS

The search of life on Mars, in the present or in the past history of the planet, is the main motivation behind the different research programs followed one another from seventies until now on the Martian surface. Our knowledge on Mars has remarkably in-

creased after the last NASA missions, especially those called Mars Explorer Rover (MER, Opportunity, Spirit and Curiosity rovers) held, from 2004 and still ongoing on the Mars landscape at Meridiani Planum and Gale Crater. Indeed, the photo reportages realized by rovers confirmed the presence of water deposits on the surface of Mars^[13].

TABLE 1 : Biogenic stromatolites vs. Abiogenic ones

	Biogenic stromatolites	Pseudostromatolites and siltstones
Lempel-Ziv index	0.46 (0.04)	0.69 (0.12)***
Geometric complexity, high and low scales	1.82 (0.02)/1.47 (0.05)	1.94 (0.02) **
Entropy, high and low scales	1.88 (0.01)/ 1.43(0.05)	1.95 (0.02)**
Tortuosity	0.79 (0.02)	0.84 (0.06)**
P		***p<0.001, **p<0.01

Nonlinear indexes are statistically different in the abiogenic pseudostromatolites and granular microspherules-containing sediments in comparison to the biogenic stromatolites ($p<0.001/p<0.01$, according to the indexes, mann-whitney test), meaning a more disordered structures than the biogenic stromatolites, biogenic stromatolites are multifractals (two fractal dimensions are present at the analyzed dimensional scales), while abiogenic stromatolites and granular microspherules-containing sediments present only one fractal dimension, at the same dimensional scales.

The presence of water is an indispensable pre-requisite for the search of life, but does not prove, of course, the presence of life itself. The search on Mars of extraterrestrial microorganisms and, in particular, of cyanobacteria, the main building materials of terrestrial stromatolites^[45]. A century of research on stromatolites has revealed diverse fabric and many structures together with a contentious history and various definitions. Besides, most of microbialites/stromatolites are carbonate in composition, but siliceous, phosphatic, iron, manganese and sulphate examples also occur^[46]. In this frame, the outcroppings on Mars surface at Meridiani Planum subjected to moderate-low diagenesis, the sulphate laminated sediments somewhere intercalated by clay containing little amount of carbonate and hematite nodules, represent an interesting field for microstructure/textures investigations^[47].

In this work we analyzed enlarged photographic or close macro images, in order to reproduce the same conditions than the Martian images obtained with the ATHENA system of the Martian Rovers, Spirit and Opportunity. At this range of magnification and resolution is possible to study microbialites microstructures and textures in the size range 0.05-1mm: a field characterized by clotted fabric, typically formed by widespread micritic clots or lumps, which may be agglutinated and set in an irregular sponge-like network structure or in intertwined turf-like communities in which the main individual components can commonly be seen with a hand-lens^[48].

From a structural point of view, in La Brava and Tebenquiche lagoons the microbial formations, developed in a brackish environment, show structures

of chaotic clusters of microspherules of various colors and dimensions, if observed in a visual field at low and high amplifications. More in detail, such microspherules are organized in complex and irregular arrays such as filaments, interwoven filaments, donuts and polispherules with an evenness of colors and dimensions. These are probable, related to the same microbial population, partially linked or simply connected by the binding and trapping activities of a microbial net organized in an Exudate Polymeric Substance (EPS), that is disappearing in diagenetic processes. This structural appearance generate peculiar texture, both chaotic and ordered one, that seems occur in terrestrial stromatolites and living microbialites, but also in the Martian laminated sediments at Meridiani Planum.

In order to search life on the Red Planet, comparing microscopically images of stromatolites on Earth and possible stromatolites photographed by the Martian Rovers, it's mandatory to be able to distinguish between true (biogenic) stromatolites and (abiogenic) pseudostromatolites^[49,50]. We show here an original morphometric approach able to perform the differential analysis at low microscopic scale (1 pixel = 0.01 mm). Our approach is based on fractal analysis^[51]. In our hands, at the Dpt. of Medical Biotechnologies of Siena (Italy), fractal analysis of histological/cytological samples has revealed itself as a very powerful method to perform differential diagnosis^[52-56].

To assess objective characterizations of biogenic stromatolites/microbialites and of abiogenic pseudostromatolites, we have carried out a quantitative fractal image analysis in order to compare, at

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the same scale of observation, images of biogenic stromatolites with the ones of abiogenic pseudostromatolites or other minerals presenting microspherules for obtaining morphometric indexes able to distinguish among them, see also^[57].

In order to distinguish between terrestrial biogenic stromatolites and abiogenic pseudostromatolites, a necessary step to look for possible biomarkers on the Red Planet, a fractal approach was performed. The former was characterized by having a multifractal texture while the latter showed a simple fractal texture with higher nonlinear indexes (more disordered structures than the biogenic ones), giving us a possible tool for differential comparison between biogenic and abiogenic samples that might be applied for astrobiological purposes. For example, in order to compare morphometrically, by fractal analysis, Martian microstructures shot by the Martian Rovers in the last ten years with the ones of terrestrial microbialites and other abiogenic stromatolites or other granular sediments, following the methodology here presented and discussed.

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REFERENCES

- [1] C.S.Cockell; Biological systems under extreme conditions: structure and function, *International Journal of Astrobiology*, **1**(2), 177 (2002).
- [2] Des D.J.Marais, B.M.Jakosky, B.M.Hynek; Astrobiological implications of Mars surface composition and properties, In: Bell JA (eds) *Planetary Science*, **9** (2008).
- [3] L.J.Rothschild, R.L.Mancinelli; Life in extreme environments, *Nature*, **409**, 1092-1101 (2001).
- [4] E.Rabbow, P.Rottberg, S.Barzyk et al.; EXPOSE – E: an ESA astrobiology mission 1.5 years in space, *Astrobiology*, **12**, 374-386 (2012).
- [5] J.H.Crowe, I.A.Haekstra, L.M.Crowe; Anhydrobiosis. *Annu.Rev.Physiol.*, **54**, 579-599 (1992).
- [6] P.Alpert; Constrains of tolerance: Why are dessication-tolerant organism so small or rare? *Journal of Experimental Biology*, **209**, 1575-1584 (2006).
- [7] E.I.Friedmann, L.Kappen, M.A.Meyer, J.A.Nienow; Long-term productivity in the cryptoendolithic microbial community of the Ross Desert, Antarctica. *Microbiol Ecology*, **25**, 51-69 (1993).
- [8] Warren K.A.Rhodes, K.L.Rhodes, S.B.Pointing et al.; Hypolithic cyanobacteria, Dry limit of photosynthesis and microbial ecology in the hyperarid Atacama Desert, *Microbiol Ecology*, **52**, 389-398 (2006).
- [9] E.I.Friedmann; Endolithic microbial life in hot and cold deserts, *Orig Life.*, **10**(3), 223-235 (1980).
- [10] E.I.Friedmann, Ocampo R.Friedmann; A primitive cyanobacterium as pioneer microorganism for terraforming Mars, *Advances in Space Research*, **15**(3), 243-246 (1985).
- [11] D.Billi, M.Baqué, H.D.Smith, C.P.McKay; Cyanobacteria from extreme deserts to space, *Advances in Microbiology*, **3**, 80-86 (2013).
- [12] F.Risacher, H.Alonso, C.Salazar; The origin of brines and salts in Chilean salars: A hydrochemical review, *Earth Science Rev.*, **63**, 249-293 (2003).
- [13] S.W.Squyres, R.E.Arvidson, J.F.Bell et al.; The opportunity rover's athena science investigation at meridiani planum, Mars. *Science*, **306**, 1698-1703 (2004).
- [14] K.Falkoner; Fractal geometry, Mathematical foundations and applications, John Wiley & Sons Ltd, (1990).
- [15] F.Kaspar, H.G.Schuster; Easily calculable measure for the complexity of spatiotemporal patterns, *Physical Review A*, **36**, 842-848 (1987).
- [16] J.Hermann, H.E.Stanley; The fractal dimension of the minimum path in two and three dimensional percolation, *J.Phys.A.*, **21**, 829-833 (1998).
- [17] P.Bevacqua; Geomorfología del salar de Atacama y estratigrafía de su núcleo y delta, Segunda Región de Antofagasta, Chile. Memoria de Título (Inédito), Universidad Católica del Norte, Facultad de Ingeniería y Ciencias Geológicas, 1-284 (1992).
- [18] H.Alonso, F.Risacher; Geoquímica del Salar de Atacama, Parte 1: Origen de los componentes y balance salino, *Andean Geology*, **23**, 113-122 (1996).
- [19] S.Cabrera, G.Pizarro; Changes in chlorophyll a concentration, Copepod abundance and UV and PAR penetration in the water column during the ozone depletion in Antarctic Lake Kitiash, *Advlimnol/Ergeb Limnol*. 1994, (1992).
- [20] L.E.Lara, R.Moreno, A.Amigo, R.P.Hobblitt, T.C.Pierson; Late holocene history of chaitén vol-

- cano: New evidence for a 17th century eruption, *Andean Geology*, **40**(2), 249-261 (2013).
- [21] Valero B.L.Garcés, C.Arenas, Delgado A.Huertas; Depositional environments of Quaternary lacustrine travertines and stromatolites from high-altitude Andean lake, Northwestern Argentina, *Can.J.Earth Sci.*, **38**, 1263-1283 (2001).
- [22] E.D.Fleming, Prufert L.Bebout; Cyanobacterial Diversity of Two High Elevation Lakes in the Atacama Desert of Bolivia, *JGR Biogeosciences*, 115 (2009).
- [23] M.E.Farías, N.Rascovan, D.M.Toneatti et al.; The discovery of stromatolites developing at 3570 m above sea level in a high-altitude volcanic lake socompa, Argentinean Andes, *PloS One*, **8**, e53497 (2013).
- [24] M.E.Farías; Contreras M Ecosistemas Microbianos; Nuevo patrimonio de la Humanidad? Revista Bitacora Ecológica 1, (Chile) (2013).
- [25] N.Stivaletta, R.Barbieri, F.Ceveninia, López García; P physicochemical conditions and microbial diversity associated with the evaporite deposits in the laguna de la piedra (Salar de Atacama, Chile), *Geomicrobiol. J* **28**, 83-95 (2011).
- [26] C.Rasuk, D.Kurth, Regina M.Flores; Microbial characterization of a gypsum endoevaporitic ecosystem in Salar de Llamara Chile, *Microbial Ecology*, 10.1007/s00248-014-0431-4 (2014).
- [27] D.Schneider, G.Arp, A.Reimer, J.Reitner, R.Daniel; Phylogentic analysis of a microbialite-forming microbial mat from a hypersaline lake of the kiritimati atoll, Central Pacific. *PLoS ONE*, doi:10.1371/journal.pone.0066662, **8**(6), e66662 (2013).
- [28] M.E.Farías, M.Contreras, M.C.Rasuk; Characterization of bacterial diversity associated with microbial mats, Gypsum evaporites and carbonate microbialites in thalassic wetlands: Tebenquiche and La Brava, Salar de Atacama, Chile. *Extremophiles*, Springer, Japan, (2014).
- [29] C.Dupraz, P.R.Reid, O.Braissant, A.W.Decho, R.S.Norman, P.T.Visscher; Processes of carbonate precipitation in modern microbial mats, *Earth Science Reviews*, **96**, 141-162 (2009).
- [30] C.Glunk, C.Dupraz, O.Braissant, K.L.Gallagher, E.P.Verrecchia, P.T.Visscher; Microbially mediated carbonate precipitation in a hypersaline lake, Big Pond (Eleuthera, Bahamas), *Sedimentology*, **58**, 720-736 (2011).
- [31] R.J.Jahnert, R.B.Collins; Characteristics, Distribution and morphogenesis of subtidal microbial systems in Shark Bay, Australia, *GSA Bulletin*, **111**, 1256-1262 (2011).
- [32] G.Arp, G.Helms, K.Karlinska, G.Scumann, A.Reimer et al.; Photosynthesis versus exopolymer degradation in the formation of microbialites on the atoll of kiritimati, Republic of Kiribati, Central Pacific, *Geomicrobiology Journal*, **29**, 29-65 (2012).
- [33] C.E.Robertson, J.R.Spear, J.K.Harris, N.R.Pace; Diversity and stratification of archaea in a hypersaline microbial mat, *Appl.Environ.Microbiol.*, **75**, 1801-1810 (2009).
- [34] T.Bosak, D.K.Newman; Microbial kinetic controls on calcite morphology in supersaturated solutions, *Journal of Sedimentary Research*, **75**, 190-199 (2005).
- [35] Van F.B.Houten, D.P.Bhattacharyya; Phanerozoic oolitic ironstones-geologic record and facies model, *Annual Review of Earth and Planetary Sciences*, **10**, 441-457 (1982).
- [36] V.Rizzo, N.Cantasano; Possible organosedimentary structures on Mars, *International Journal of Astrobiology*, **8**(4), 267-280 (2009).
- [37] V.Rizzo, N.Cantasano; Textures on Mars: Evidences of a biogenic environment, In: *Memorie della Società Astronomica Italiana*, **82**(2), 348-357 (2011).
- [38] L.J.Rothchild; Earth analogs for martian life: Microbes in evaporates, A new model system for life on Mars, *Icarus*, **88**, 246-260 (1990).
- [39] G.Arrhenius, S.J.Mojzsis; Extraterrestrial life – life on Mars, Then and now, *Current Biology*, 1996, **6**, 1213-1216 (1990).
- [40] R.A.Kerr; Putative martian microbes called microscopic artifacts, *Science*, **278**, 1706-1707 (1997).
- [41] H.Vali, J.Paquette, S.K.Sears et al.; Significance of microstructures for the recognition of biological mediated mineralization, *Proc.SPIE*, **3111**, 137-143 (1997).
- [42] F.Westal; The nature of fossil bacteria: A guide to search for extraterrestrial life, *J.Geophys.Res.*, **104 E7**, 16437-16451 (1999).
- [43] P.J.Boston, M.N.Spilde, D.E.Northup et al.; Cave biosignature suites: Microbes, Minerals and Mars, *Astrobiology Journal*, **1**(1), 25-55 (2001).
- [44] M.R.Walter, Des D.J.Marais; Preservation of biological information in thermal spring deposits – developing a strategy for the search for fossil life on Mars, *Icarus*, **101**, 129-143 (1993).
- [45] R.P.Reid, P.T.Visscher, A.W.Decho, J.F.Stolz, B.M.Bebout, C.Dupraz, I.G.Macintyre, H.W.Paerl, L.Prufert-Bebout, T.F.Steppe, Des D.J.Marais; The role of microbes in accretion, Lamination and early lithification of modern marine stromatolites, *Nature*, **406**, 989-992 (2000).

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- [46] R.Riding; The nature of Stromatolites, 3,500 Million years of history and a century of research, In: *Advances in Stromatolite Geobiology*, Reitner, J et al (Editions), *Lecture Notes in Earth Sciences*, **131**, 29-74 (2011).
- [47] J.P.Grotzinger, D.Beaty, G.Dromart et al.; Mars sedimentary Geology: Key concepts and outstanding questions, *Astrobiology*, **11**(1), 77-87 (2011).
- [48] R.Riding; Microbial carbonates: The geological record of calcified bacterial-algal mats and biofilms, *Sedimentology*, **47**(1), 179-214 (2000).
- [49] N.McLoughlin, L.A.Wilson, M.D.Brasier; Growth of synthetic stromatolites and wrinkle structures in the absence of microbes – implications for the early fossil record, *Geobiology*, **6**, 95-105 (2008).
- [50] R.Riding; Abiogenic and hybrid authigenic carbonate crusts: Components of Precambrian stromatolites, *Geological Croatica*, **61**, 2-3, 73-103 (2008).
- [51] B.Mandelbrot; *The Fractal Geometry of Nature*, W.H.Freeman & Co., (1982).
- [52] G.Bianciardi, C.Miracco, De M.M.Santi, P.Luzi; Differential diagnosis between mycosis fungoides and chronic dermatitis by fractal analysis, *J Dermatol Sci.*, **33**, 184–186 (2003).
- [53] G.Bianciardi, I.Tanganelli, D.Totangiancaspro, M.Brogi, A.Carducci, M.M.De Santi; Fractal analysis of monocytes, In: *Diabetes, Clin Hemorheol Microcirc*, **35**(1-2), 269- 272 (2006).
- [54] G.Bianciardi, C.Traversi, R.Cattaneo et al.; Phase transition of the Microvascular Network, *Architecture in Human Pathologies*, *Theor Biol.Forum.*, **105**(1), 37- 45 (2012).
- [55] G.Bianciardi, C.Miracco, S.Lazzi, P.Luzi; Fractal analysis of epithelial-connective tissue in basal cell carcinoma of the skin, *Current Bioinformatics*, **8**, 357-361 (2013a).
- [56] G.Bianciardi, M.Acampa, I.Lamberti et al.; (Microvascular abnormalities in Rett syndrome, *Clin Hemorheol Microcirc*, **54**, 109-113 (2013b).
- [57] Storrie M.C.Lombardi, F.A.Corsetti, P.Grigolini et al.; Complexity analysis to explore the structure of ancient stromatolites, *Chaos, Solitons & Fractals*, **20**(1), 139-144 (2004).